

INTRODUCTION

The flavor of fresh fluid milk is difficult to describe because basically milk is a bland product. From a gustatory standpoint, it can have either a slight sweet character as a result of its lactose content, or a slight salty character due to the presence of chloride salts. Fresh milk imparts a pleasant smooth sensation to the mouth and there should be no evidence of astringency (Keeney 1961). A basic olfactory quality, characteristic of all fresh milks, exists, but the normal intensity of the flavor is such, that for all practical purposes, milk has very little flavor. This is not to suggest that all milks are flat since the flavor of milk is highly susceptible to change as a result of farm and manufacturing practices, in addition to changes which occur spontaneously in the milk itself. Off-flavors resulting from these practices and spontaneous changes, although abnormal, are eventually accepted by the consumer as the normal flavor when their presence and intensity is consistent. It is only when the intensity of the normal flavor changes or the sudden presence of other flavors appear that the consumer objects. The dairy manufacturer is confronted, therefore, with the problem of marketing a product with uniform flavor, whether it be free of or contain slight off-flavors. A knowledge of the characteristic flavor and the off-flavors which can arise, including the conditions conducive to formation, are of utmost importance to the dairy industry.

CHARACTERISTIC FLAVOR OF MILK

At present, it is not definitely known what compounds contribute to the characteristic flavor of milk. Low molecular weight compounds present in trace amounts such as acetone, acetaldehyde, butyric acid, and certain other low molecular weight fatty acids have been suggested (Jenness and Patton 1959) as contributors to the flavor. Methyl sulfide, shown to be a constituent of fresh milk and detectable on cows breath, imparts a flavor highly characteristic of fresh milk (Patton *et al.* 1956). Other low molecular weight compounds identified in fresh milk include formaldehyde, butanone-2, pentanone-2, hexanone-2, and heptanone-2 (Wong and Patton 1962). Studies not related to the characteristic flavor, but employing fresh milk as control have resulted in the identification of the C_3 to C_{12} normal, saturated aldehydes and the C_{13} to C_{16}

normal and branched chain saturated aldehydes (Parks *et al.* 1963; Wishner and Keeney 1963). At the concentrations detected, the individual aldehydes would not appear to affect the overall flavor of the product, but collectively their effect remains to be determined.

Several flavor compounds identified in fresh dairy products must also be given some consideration as to their contribution to the characteristic flavor of fresh fluid milk. Among these compounds are (1) diacetyl—an accepted flavor constituent of fresh butter (Jenness and Patton 1959), (2) isovaleraldehyde—observed in fresh cream (Wong 1963), (3) 4-*cis*-heptenal—reported to impart a creamy flavor to fresh butter (Begemann and Koster 1964), and (4) δ -lactones—flavor constituents of butter and shown to occur in fresh cream (Boldingh and Taylor 1962) and pasteurized milk (Patton 1961).

Most flavors, at least in dairy products, cannot be attributed to one compound, but rather to a mixture of compounds; whereas one compound will show different flavor characteristics at various concentrations, mixtures of compounds should accentuate this condition. It is significant that the compounds which have been implicated in the characteristic flavor of milk have also been observed in higher concentrations in many of the off-flavors which arise in milk. For example, methyl sulfide at concentrations distinctly above threshold imparts a cowy flavor to milk (Patton *et al.* 1956), an off-flavor which has also been associated with high concentrations of ketones (Josephson and Keeney 1947). Observations, such as these, must be considered when defining the characteristic or normal flavor of a bland product which is subject to various off-flavors. Some of the off-flavors of milk and how they arise to alter the flavor is the main topic of this presentation.

FEED FLAVORS

It was recognized as early as 1757 (Bradley 1757) that the feed consumed by the cow was a contributing cause of abnormal flavors in milk and the literature contains literally hundreds of publications and several comprehensive reviews on the subject (Strobel *et al.* 1953; Babcock 1938). The role of feeds in the flavor of milk does not, however, follow a standard pattern. The early literature clearly indicates that milk produced on certain feeds contains flavors characteristic of the feed consumed, while other feeds impart flavors which are not characteristic of the feed. Furthermore, certain highly flavored feeds do not impart flavors, or only marginal flavors, whereas some lightly flavored feeds impart strong off-flavors.

Feed flavors can be controlled to a certain extent by proper feeding practices such as removing animals from pasture a few hours prior to

milking, feeding highly flavored feed immediately after milking, keeping barns well ventilated, providing high quality feeds essentially free of weeds, etc. (Jenness and Patton 1959; Babcock 1938). Although these practices have lessened, to a great extent, the intensity of feed flavors in milk, they do not in all cases prevent their appearance. It is quite evident that the feed consumed and the animals' physiological processes influence the final flavor.

Recently, a Cornell team conducted a series of experiments (Dougherty *et al.* 1962; Shipe *et al.* 1962) which demonstrated the role of three pathways in the transfer of feed flavors to milk. Two animals were fitted with tracheal and ruminal fistulae and the experiment was so designed that flavor substances under study could be added directly into the rumen or into the lungs. Furthermore, eructated gases from the rumen were allowed to pass into the lungs or prevented from doing so by passing fresh air into the tracheal fistula. The results of this study are summarized in Table 41. When the vapors of an onion slurry were intro-

TABLE 41
TRANSMISSION OF FLAVOR FROM ONIONS TO MILK¹

Onion slurry → Tracheal cannula → lungs	No flavor up to 90 min.
Fresh air → Tracheal cannula	Flavor intensity low up to 45 min.
Onion slurry → Ruminal cannula	
Cow breathing normally	
Onion slurry → Ruminal cannula	Pronounced flavor in 15 min.
Onion slurry incubated 30 min. at 37°C. → lungs with rumen ingesta	Pronounced flavor in 15 min.
Rumen ingesta → lungs	No flavor up to 90 min.

¹ Dougherty *et al.* (1962).

duced into the trachea, no detectable off-flavor was observed in the milk after 90 minutes. When the slurry was introduced into the rumen and eructated gases prevented from entering the lungs, a low level of flavor was detected in the milk. A pronounced flavor occurred in the milk in a short period of time when the eructated gases were not excluded from the lungs. The role of the rumen in liberating flavor substances from onions which are transmitted to the milk becomes evident when the onion slurry is incubated with rumen ingesta and the vapors then passed through the tracheal fistula into the lungs. It must be noted here, that the off-flavor which occurred in the milk was not characteristic of the onion. Similar experiments on other substances gave rise to off-flavors which were rather typical of the feed. Employing the same techniques, the introduction of various low molecular weight alcohols, esters, aldehydes and ketones, compounds identified in the

volatiles of grass and corn silage (Morgan and Pereira 1962A and B) resulted in off-flavors in the milk. There was no apparent difference between the methods of transmission, although it was observed that the flavors appeared in the milk much more rapidly when introduced into the lungs than through the digestive system. It must be noted that although, with a few exceptions the compounds administered in these studies gave rise to off-flavors, it was necessary to introduce 25 to 75 ml. of most of the compounds to attain these off-flavors. Honkanen and co-workers (Honkanen *et al.* 1964) have carried out similar experiments which are unique in several respects. The most unique aspect of their work is that the animals are producing milk on an odorless, purified diet consisting of cellulose, starch, sucrose, urea, inorganic ammonium salts, minerals, Vitamins A and D, and maize oil (Virtanen and Lampila 1962). The animals reportedly produce milk with the characteristic milk flavor, but more important, free of off-flavors as a result of feeds and feeding practices. Hence, the product is ideally suited for studying the transfer of flavor and potential flavor compounds into milk. The results of studies in which 1 to 2 gram quantities of various compounds were added to the rumen and recovered from the milk by vacuum distillation techniques and analyzed quantitatively by gas chromatography are reported in Table 42. Although the percentage transfer is low, the authors conclude that as a result of an additive effect, some classes of compounds can give distinct off-flavors to the milk. The results are interesting in many respects, since they may explain why certain feeds do not impart off-flavors to milk while others impart flavors which are not characteristic of the feed consumed. As can be observed, aliphatic alcohols with an odd-number of carbon atoms are transferred to the milk at a greater degree than others. Of the ethyl esters administered, only those with an even number of carbon atoms in the fatty acids enter the milk while the methyl esters are not transferred in detectable amounts. Only traces of the aldehydes administered, with the exception of 2-methylpropanal, were transferred. The maximum concentration of the higher molecular weight compounds is reached in the milk after a longer period of time following administration of these compounds. This latter observation may explain the differences in time elapsed between feeding and the presence of detectable off-flavors which occur when different feeds are consumed.

As mentioned previously, those methods employed to prevent or lessen the effect of feeds on the flavor of milk are, for the most part, those which lengthen the time between feeding and milking. Petersen and Brereton (1942), following experiments on the inhalation of various compounds, suggested that an equilibrium of flavor compounds from

TRANSFER OF SOME ALIPHATIC COMPOUNDS TO MILK VIA THE RUMEN¹

Substance	Amount Fed, Gm.	Con. at the Maximum, $\mu\text{g./liter Milk}$	Total Amount Transferred to Milk, $\mu\text{g.}$	Percentage of Total Amount Fed
Alcohols				
<i>n</i> -Pentan-1-ol	2	380	250	0.013
<i>n</i> -Hexan-1-ol	2	30	20	0.001
<i>n</i> -Heptan-1-ol	2	120	100	0.005
<i>n</i> -Octan-1-ol	2	?
<i>n</i> -Nonan-1-ol	2	75	100	0.005
<i>cis</i> -Hex-3-en-1-ol	2	45	25	0.0013
<i>trans</i> -Hex-3-en-1-ol	3	25	15	0.0005
<i>dl</i> -Oct-1-en-3-ol	1	20	15	0.0015
Aldehydes				
2-Methyl-propanal	2	1500	600	0.03
Hexanal	2	15	10	0.0005
Heptanal	2	20	10	0.0005
Octanal	2	30	10	0.0005
Ketones				
Pentan-2-one	2	1300	1000	0.05
Hexan-2-one	2	1100	800	0.04
Heptan-2-one	2	750	250	0.013
Octan-2-one	2	490	150	0.0075
Nonan-2-one	2	150	50	0.0025
Decan-2-one	2	60	30	0.0015
Undecan-2-one	2	40	25	0.0013
Octan-3-one	2	50	25	0.0013
Oct-1-en-3-one	2
Esters				
Methyl esters of C ₆ -C ₁₀ fatty acids	2
Ethyl esters of C ₅ , C ₇ , C ₉ fatty acids	2
Ethyl butanoate	2	120	120	0.006
Ethyl hexanoate	2	60	60	0.003
Ethyl octanoate	2	120	100	0.005
Ethyl decanoate	2	120	100	0.005
Butyl 2-methyl-propanoate	1	300	160	0.016
Butyl butanoate	1	160	120	0.012
Butyl 3-methyl-butanoate	1	140	90	0.009
Butyl pentanoate	1	200	150	0.015
Butyl hexanoate	1	260	170	0.017
Butyl octanoate	1	110	75	0.0075

¹ Honkanen *et al.* (1964).

feeds exists between the lungs, blood, and milk. In time, the concentration in the lungs becomes low, shifting the equilibrium away from the milk; hence, decreasing the concentrations of these compounds in the final product.

RANCID FLAVOR

All raw milks are susceptible to the development of rancid flavors as a result of the liberation of fatty acids by lipases, enzymes native to milk.

At least two different lipase systems have been reported (Tarassuk and Frankel 1957) in milk, one irreversibly adsorbed on the fat globule membrane when freshly drawn milk is cooled and the other associated with the casein in the milk plasma. However, as many as four systems have been indicated (Gutfreund 1963). Despite the differences with regard to the number of systems which are present, one of which has been recently isolated (Chandran and Shahani 1963), it is obvious from the literature, that more than one system is operative.

The appearance of rancid flavor in market milk seems to follow a seasonal pattern. The greatest incidence of the off-flavor has been reported to occur in the late fall and early winter months (Hileman and Courtney 1935). This seasonal trait has been correlated with late stages of lactation and the practical observation that green pastures decrease and dry feeds increase the incidence of the off-flavor (Tarassuk *et al.* 1962).

Basically, raw milks can be classified into either one of two groups with regard to lipase activity, (1) those which develop rancid flavors spontaneously without any treatment other than cooling of the freshly drawn milk, and (2) those which develop rancid flavors only after activation treatments (Schwartz 1965). Nonspontaneous lipolysis occurs in raw milk by several activation treatments. These include, (a) excessive agitation, (b) homogenization, (c) separation or clarification, (d) warming cold milk to 27°C. or 32°C. and recooling to lower temperatures, (e) freezing and thawing, and (f) the addition of small amounts of raw milk to pasteurized, homogenized milk (Jenness and Patton 1959). Since pasteurization destroys the lipases of milk, the problem of rancidity is really one of handling the raw product. In recent years, the problem of hydrolytic rancidity has been aggravated by modern trends of handling raw milk, some of which are the pumping of milk from the milking machine to the holding vats through pipelines (Speer *et al.* 1958), the use of milk measuring devices in pipelines (Janzen 1963), and bulk tank hauling of milk (Jenness and Patton 1959) all of which create varying degrees of agitation.

Milkfat is unique among fats in that it contains substantial amounts of low molecular weight fatty acids. It has been suggested (Jenness and Patton 1959) that the release of these lower acids, especially butyric acid, imparts to milk its characteristic rancid flavor. There is available in the literature, indirect support for this contention. It has been demonstrated that butyric acid is a major constituent liberated from milkfat by lipase activity (Harper 1955). It was originally suggested by Harper that milk lipases showed a specificity for liberating butyric acid from milkfat triglycerides. Recent work has demonstrated, how-

ever, that the enzymes show a specificity for the alpha position of triglycerides (Gander and Jensen 1960) and most of the butyric acid is esterified in the alpha position (Jensen *et al.* 1960). Nevertheless, with our present knowledge of the additive effect of compounds in the same class on the flavor of a product (Day *et al.* 1963), it is difficult to ascribe the off-flavor to one particular fatty acid.

OXIDIZED FLAVOR

If any uncertainty remains that the milks from individual animals vary in their susceptibility to the spontaneous development of off-flavors, such doubt is removed when one considers the development of oxidized flavor in fluid milk. The freshly drawn milk of some animals develops an off-flavor within hours after cooling while that of others resists oxidative deterioration even though relatively large amounts of copper have been added to the product. Investigators have gone so far as to show (Guthrie and Brueckner 1934) that the milks from the individual quarters of the same animal differ in their susceptibility to oxidative deterioration. It has been reported (Potter and Hankinson 1960) that as many as 23% of the animals in a herd will produce milk which will develop an oxidized flavor within 24 to 48 hours storage and as was the case with rancid flavor, the incidence of the off-flavor increases when the animals are on dry feeds and decreases during pasture feeding (Greenbank 1948)—reportedly due to the differences in the antioxidative properties of the feeds (Krukovsky 1961).

It is not within the scope of this presentation to review the literature regarding the mechanisms involved in this phenomenon, since recent reviews on the subject are available (Riel 1952; Patton 1962; Parks 1965). It has been demonstrated (Greenbank 1940) that a correlation exists between the appearance of the off-flavor and conditions favoring a mild oxidation. The resistance of some milks to undergo oxidative deterioration, even in the presence of added copper, has been explained on its resistance to change in the oxidation-reduction potential (Greenbank 1940).

The site of oxidation in fluid milk is the phospholipids associated with the fat globule membrane (Smith and Dunkley 1959) and indeed the inhibition of spontaneous autooxidation in fluid milk is accomplished by homogenization which physically alters the membrane surrounding the fat globules. The constituents of milk which have been implicated in the mechanism include the ratio of ascorbic to dehydroascorbic acid, natural copper complexed with the proteins in the fat globule membrane, and the phospholipids and oxygen dissolved in the system.

The role of enzymes in the mechanism has been proposed on several

occasions (Kende 1932; Aurand and Woods 1959) and rejected on just as many (Smith and Dunkley 1960 and 1962). Recently, Patton (1962) has compared the system previously described to an enzyme system and suggests that the controversy of enzymatic vs. nonenzymatic is a question of semantics regarding the definition of an enzyme.

TABLE 43
QUANTITATIVE CARBONYL ANALYSES ON THE BUTTEROIL FROM NONOXIDIZED AND SPONTANEOUSLY OXIDIZED FLUID MILK¹

Carbonyl	Concentration in Parts Per Billion of 4% Milk	
	Nonoxidized Milk	Spontaneously Oxidized Milk
Pentanal	1.46	2.25
Hexanal	2.38	13.48
Heptanal	6.95	6.04
Octanal	1.84	1.95
Nonanal	1.65	3.04
Decanal	0.97	1.12
Undecanal	0.31	0.27
Dodecanal	0.74	2.06
C ₁₃ -C ₁₆ Sat. Ald.	8.90	11.25
2-Hexenal	...	0.09
2-Heptenal	...	0.39
2-Octenal	0.19	0.55
2-Nonenal	0.24	0.62
2-Decenal	0.59	1.17
2-Undecenal	0.58	1.08
2,4-Nonadienal	...	0.39
2,4-Decadienal	0.17	0.83
2,4-Undecadienal	...	0.12
2,4-Dodecadienal	...	0.22

¹ Parks *et al.* (1963).

Table 43 represents the carbonyl compounds identified and quantitized in the fat of nonoxidized and spontaneously oxidized fluid milk as judged by a 10 member taste panel. As one might expect, the compounds identified do not differ significantly from those reported in other oxidized dairy products (Forss *et al.* 1955; El-Negoumy *et al.* 1961). The differences in flavor of the two products can be attributed, for the most part, to the concentrations of some of the individual compounds. Although the compounds identified in the control did not impart an oxidized flavor to the milk, one cannot conclude that the carbonyls did not influence the flavor, which was judged to have a definite feed flavor.

SUNLIGHT FLAVOR

When milk is exposed to direct sunlight, for even very short periods of time, an off-flavor develops which has been described as resembling

cooked cabbage and burning hair. No one deliberately exposes milk to sunlight, but this can conceivably occur when glass bottled milk is delivered at the home doorstep or allowed to set out on the kitchen table in full view of the sun's rays. Although direct sunlight is the most effective catalyst of the off-flavor, incandescent or fluorescent light to which milk is frequently exposed during handling, processing, and storage can be contributive (Smith and MacLeod 1955). The early literature refers to the flavor defect under the general term of sunlight, but actually two distinct flavors occur in whole milk exposed: an oxidized flavor as a result of lipid oxidation and an activated flavor which originates in the proteins of milk.

The carbonyls in whole milk exposed to sunlight have been reported (Wishner and Keeney 1963) and they do not, for the most part, differ qualitatively from those reported for spontaneously oxidized milk or copper induced oxidation of skim milk. The authors do not report the presence of 2,4-dienals in the sunlight exposed milk but this may be the result of the size of samples analyzed (500 ml.).

The activated flavor has been shown to originate in the proteins of milk and is dependent, although not entirely, on the presence of riboflavin (Flake *et al.* 1940; Patton 1954). The off-flavor has been attributed to the reaction of methionine with riboflavin and somewhat of a controversy exists as to the compound or compounds which arise to impart the characteristic flavor. Methional, as a result of its formation from methionine by the Strecker degradation and the similarity in flavor with that of exposed milk was originally proposed as the compound responsible for the defect (Patton and Josephson 1953; Patton 1954). The presence of methional could not be confirmed, however, in studies on the carbonyls in sunlight exposed milk (Wishner and Keeney 1963) despite methods capable of accomplishing this. Ballance (1961) has reported that methional is further degraded to methyl mercaptan, dimethyl sulfide and acrolein during the Strecker degradation of methionine. Samuelsson (1961 and 1962), although acknowledging that methional can result in milk by the Strecker degradation of free methionine, reported that the aldehyde is not liberated when methionine is present in a peptide linkage. Instead, highly flavorful mercaptans, sulfides, and disulfides were shown to result from the irradiation of model peptides.

Recently, it has been reported (Zittle 1963; Zittle *et al.* 1964) that a loss of tryptophan, histidine, tyrosine, cystine, and methionine accompanies photo-oxidation of β -casein and α_s -casein. This suggests that flavor compounds, in addition to those derived from methionine, may play a significant role in the activated flavor of milk.

Heat Treatment	O-R (Volts)	Flavor	Nitroprusside Reaction
None	0.2441	Normal	—
62.8°C., 30 min.	0.2423	Normal	—
71.1°C., momentarily	0.2426	Normal	—
76.7°C., momentarily	0.2349	Cooked ±	±
82.2°C., momentarily	0.2331	Cooked ++	+
87.8°C., momentarily	0.2025	Cooked ++++	++++

¹Josephson and Doan (1939).

HEATED FLAVORS

With few exceptions, all market milk is pasteurized at 61.7°C. for 30 minutes by the holding process or 71.7°C. for 15 seconds in the high-temperature, short-time process. An organoleptic comparison of milk before and after pasteurization readily demonstrates that the treatment is not without effect on the flavor of the product. Although little is known with regard to the changes which occur at pasteurizing temperatures, it is to be expected that the loss of volatile compounds is as much a contributing factor as is the formation of new heat generated flavor constituents.

When milk is heated to approximately 74°C. or higher, even momentarily, a distinct cooked flavor develops (Gould and Sommer 1939; Josephson and Doan 1939) the intensity of the flavor being a function of the temperature and duration of heating. The flavor is the result of volatile sulfides, hydrogen sulfide in particular (Jenness and Patton 1959), which are liberated from the activated sulfhydryl groups of β -lactoglobulin and to a lesser extent the proteins of the fat globule membrane (Hutton and Patton 1952). Table 44 demonstrates the effect of various heat treatments on sulfhydryl activity as measured by the nitroprusside test and the appearance of a cooked flavor. Accompanying the appearance of a cooked flavor, is a lowering of the oxidation-reduction potential of the system—a change which is beneficial when one considers the keeping quality of sterile milks.

As the heat treatment is prolonged at 74°C or increased to higher temperatures, the cooked flavor, at some undetermined point, gives way to a caramelized flavor and the initiation of Maillard type reactions take place.

DRY AND STERILE CONCENTRATED MILKS

Since World War II, considerable research has been conducted on preparing dry and sterile concentrated milks as a means of storing milk

for future consumption, as a reconstituted fluid beverage. The major goal is to produce and maintain a product which, when reconstituted will have a flavor typical of fresh fluid milk. Various problems accompany such products, not the least important of which is changes which occur in the flavor as a result of heat processing and storage conditions.

Dry whole and sterile concentrated milks have much in common with regard to flavor changes which occur initially and during storage. However, several differences do exist. Concentrated milks, as a result of a need for sterilization, are subjected to more intense heat treatments than are dry milks, a treatment which renders the product more conducive to cooked and other off-flavors accompanying the Maillard reaction, but less susceptible to oxidative deterioration, a result of the reducing conditions which exist in cooked products. Dry milks, on the other hand, are prepared with heat treatments which minimize the development of heat induced off-flavors, making it, however, susceptible to oxidative deterioration. Heat treatment alone does not govern the susceptibility to oxidative deterioration or Maillard-type reactions. One must also take into account that one product exists in the dry state and the other in a concentrated liquid state. The problem of oxidized flavor in dry milks is academic, since the oxidative mechanism can be controlled by packaging the product in an atmosphere essentially free of oxygen.

Conventional evaporated milk is normally sterilized at 117°C. for 15 minutes, which is a substantial amount of heat regardless of what the product is. Recent trends, in an effort to minimize the effects of heat on the product, have been to sterilize at temperatures approaching 148°C. for one or two seconds. The effect of the higher temperature for a short

TABLE 45
FLAVOR COMPOUNDS IDENTIFIED IN VARIOUS DRY AND STERILE CONCENTRATED MILKS

Acetone	δ -decalactone	Formaldehyde
Butanone-2	δ -dodecalactone	Acetaldehyde
Pentanone-2	δ -tetradecalactone	Propanal
Hexanone-2	γ -dodecalactone	Pentanal
Heptanone-2		Hexanal
Nonanone-2		Heptanal
Undecanone-2	Caprylic acid	Octanal
Tridecanone-2	Caproic acid	Nonanal
Pentadecanone-2	Capric acid	Decanal
	Lauric acid	Dodecanal
	Myristic acid	Tetradecanal
	Palmitic acid	
Furfural	Vanillin	
Hydroxymethyl furfural	Benzaldehyde	
Diacetyl	<i>o</i> -Aminoacetophenone	
Maltol		
Methylpropanal		
3-Methylbutanal		

period of time is an easily noticeable improvement in the fresh sterilized product, and for the most part, this improvement continues throughout the storage life.

This is not to suggest that the newer products do not undergo flavor deterioration since other mechanisms, not related to the initial heat treatment of the product are still operative.

Table 45 lists the flavor compounds which have been reported in stale dry and sterile concentrated milks. The products, in which these compounds have been identified, were prepared under various manufacturing conditions and the table is presented only to demonstrate the apparent complexity of the reactions which can occur in stored dairy products.

The presence of the C₅ to C₁₅ odd-numbered carbon methyl ketones and a series of lactones has been demonstrated in various studies concerned with the heat treatment and storage stability of milkfat and fat containing dairy products. Lactones have been observed in pasteurized milk and along with the methyl ketones are known flavor constituents of dry whole milks and sterile concentrated milks (Patton 1961; Keeney and Patton 1956; Parks and Patton 1961; Muck *et al.* 1963; Wong *et al.* 1958). In the latter products, the concentrations increase during storage and their contributions to the so-called stale flavor of these products have been adequately established. At present, only the C₁₀, C₁₂, and C₁₄ δ -lactones and the C₁₂ γ -lactone have been demonstrated in these products, but various other lactones are known to occur in heated milkfat (Parliment and Nawar 1965; Boldingh and Taylor 1962).

The mechanisms involved in the formation of butanone-2 and hexanone-2 have not been determined although the evidence clearly indicates that they originate from mechanisms completely divorced from the other methyl ketones. The presence of butanone-2 in gamma and solar irradiated skimmilk (Day *et al.* 1957; Wishner and Keeney 1963) has led to speculation that the ketone may arise as the result of a branched alkyl free radical derived from leucine. Hexanone-2 has recently been reported in sterile concentrated milks (Bingham 1964) and substantial quantities were observed in several dry whole milks examined (Parks and Patton 1961).

The presence of a homologous series of saturated aldehydes is not unexpected since they have been observed in fresh fluid milk. However, on the basis of the concentrations observed in dry milks and control samples (Parks and Patton 1961; Bassette and Keeney 1960), the majority of these aldehydes obviously occurred as the result of autoxidation of lipid material.

The presence of free fatty acids (Muck *et al.* 1963) is also to be expected since one would predict a certain amount of hydrolysis to take

place over a period of time. Some thought has been given to the re-activation of lipases in sterile milks, but there is no evidence available to support this hypothesis.

Furfural, hydroxymethylfurfural, diacetyl, maltol, methyl propanal, and 3-methylbutanal (Parks and Patton 1961; Bassette and Keeney 1960; Potter and Patton 1956) are directly or indirectly the result of the heat treatment employed in preparing these products. Methyl propanal and 3-methylbutanal can result from the Strecker degradation of the amino acids valine and leucine while hydroxymethylfurfural, furfural, diacetyl, and maltol are known products of sugar fragmentation and dehydration during the Maillard reaction (Hodge 1953).

The identification of vanillin (Cobb *et al.* 1963) in commercial sterile concentrates suggests an interesting aspect of flavor development in dairy products. On the basis of its structure alone, it is difficult to assign a precursor among the normal constituents of milk to this highly flavorful compound. It has been reported that vanillin is a product of a mild oxidation or alkaline degradation of lignin (Richter 1952). Therefore, the possibility exists that some ligneous fragment or fragments from feed passes through the metabolic processes of the cow and finds its way into the milk. Heat treatments such as employed in sterilization or drying of milk may then give rise to this compound and contribute to the caramelized flavor. We have observed on occasions some fresh, pasteurized fluid milks with flavors which were highly suggestive of vanillin.

Benzaldehyde (Parks and Patton 1961) is another compound which may arise in dairy products as a result of feed constituents entering milk; this compound has been observed in both grass and corn silage (Morgan and Pereira 1962A and B).

o-Aminoacetophenone is one of the latest flavor compounds to be identified in dry and sterile concentrated milks (Parks *et al.* 1964). The synthetic compound has a grape-like odor and imparts a characteristic flavor to milk. The flavor threshold concentration of this compound has been determined to be in the order of 0.5 parts per billion in skim milk. Little is known with regard to the mechanisms involved in the formation of this compound in dairy products. It has been reported to result from the alkaline degradation of tryptophan and kynurenine in model systems (Spacek 1954). Indican (indoxyl sulfate) has been suggested as a possible source of the flavor compound. The latter compound has been found in milk (Spinneli 1946) while the presence of kynurenine has not been established.

On the initial assumption that the compound may arise in dry milks as the result of high heat treatments, we prepared (Parks *et al.* 1965) a series of milk powders from skim milk heated to temperatures ranging

from 76.7° to 143.3°C. for fifteen seconds. However, we were not able to detect organoleptically any significant differences in the samples initially or after four months storage at room temperature when packed in either nitrogen or air. In addition to the flavor studies, we analyzed the original heated milks to determine the quantities of *o*-aminoacetophenone which could result from the alkaline degradation of the basic fraction of the nonprotein nitrogen compounds in milk by a modification of the method of Spacek (1954). The results of that study are reported in Table 46. The amount of *o*-aminoacetophenone which arises decreases

TABLE 46

RELATIVE AMOUNTS OF *o*-AMINOACETOPHENONE LIBERATED FROM THE BASIC FRACTION OF THE NONPROTEIN NITROGEN OF 500 ML. OF SKIMMILK HEATED TO VARIOUS TEMPERATURES

Heat Treatment °C.	O.D. at 353 m μ in 10 ml. of Hexane ¹
Raw milk	0.034
76.7°C., 15 sec.	0.039
93.3°C., 15 sec.	0.034
110°C., 15 sec.	0.026
126.7°C., 15 sec.	0.021
143.3°C., 15 sec.	0.008

¹ Average reading of four determinations.
Parks *et al.* (1965).

gradually as the milk is heated over 93.3°C. It is quite difficult to interpret these results at this time since we were not successful in generating the flavor compound to any significant degree, if at all, in the dry milks prepared. The data may reflect nothing more than the loss of free tryptophan in the milk as a result of the Maillard reaction during heating. Then again, the possibility exists that the heat treatment of the milk and the nonprotein nitrogen are not involved in the reactions leading to the presence of this compound in dairy products. We have observed that the concentration of this flavor compound appears to vary among commercial sterile concentrates despite similar temperatures employed in the sterilization treatment.

Milk as evidenced by the brief review of literature presented herein can have many flavors. In fact, milk free of off-flavor is the exception rather than the rule. Most slight off-flavors are not objectional to the consumer, although a few such as oxidized are more so than others. In fact, it is not uncommon to hear individuals say they like a slight rancid or a slight feed flavor in their milk. The consumer's rejection of dry and sterile concentrated milks can be traced to the rather pronounced off-flavors which develop in these products during storage despite the convenience of having such products available in the home at all times. Up until such time that the initial flavor of these products can be main-

tained over a period of time, dry and sterile concentrated milks will not be used as a fluid beverage to any degree. To quote Jenness and Patton (1959), "A food may be wholesome, nutritious, attractively packaged and reasonably priced, but if its flavor is poor, it will be rejected by the consumer."

DISCUSSION

W. F. Wilkens.—Is lipase destroyed by the flash pasteurization treatment of 148°C. for 1 to 2 seconds?

O. W. Parks.—I believe it would probably remain active.

A. M. Swanson.—There is definite evidence that you can get heat reactivation of lipolytic activity with high-temperature, short-time heat treatments.

R. Self.—I was very interested in the speculation that vanillin may arise from lignin. In consideration of some work by Goheen on commercial forestry products, where he studies the demethylation reactions, it would be interesting if you could also find dimethyl sulfide as a degradation product with vanillin.

W. F. Shipe.—Do you consider the development of the activated flavor as an oxidative process?

O. W. Parks.—It is to a certain extent, whether it is a completely oxidative reaction I couldn't say.

C. Wiener.—Do the plasmalogens actually play a role in the release of aldehydes to produce off-flavors in milk? Also are there enzyme systems present that release these aldehydes, or do you attribute the release to a rise in acidity of the milk. Have other aldehydes beside the C₁₆ and C₁₈ been found?

O. W. Parks.—The only place I know that the plasmalogens supposedly play a role is in the irradiation of milkfat. In most fresh milks there are free saturated aldehydes.

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